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GENETIC AND ENVIRONMENTAL VARIATION IN *PIERIS BRASSICAE*

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INTRODUCTION

THE PRINCIPAL FORM OF VARIATION in *Pieris brassicae* L. consists of either a reduction or expansion in the amount of black present. In certain local populations the variation is constant and marked enough to be designated as a race, such as *P. brassicae cheiranthi* in the Canary Islands. Marked variation in color, however, is exceedingly rare and, until now, only some half dozen specimens were known.

In 1950 a stock of *P. brassicae* originating from Cambridge was established at the Unit of Insect Physiology and has been continuously bred ever since. This stock has already been reported on (David & Gardiner 1951; David 1957). In 1959 another stock, originating from Oxford, was established as a separate culture and was also continuously brooded under the same conditions as the Cambridge stock.

Until 1960 the amount of variation noticed in the Cambridge stock was small, and confined to known variation in the black markings. This has already been briefly reported on (David & Gardiner 1961).

During the past three years, however, two color varieties have appeared, as well as the previously unknown albino form; known varieties also seem to have been more common, although this could well be due to more careful observation (fig. 1).

The opportunity was taken to determine the genetics of these varieties and also to examine the combination of two pairs of allelomorphs to produce a double recessive. It is believed that this is the first time such a cross has been done with a butterfly.

From time to time specimens considerably smaller than the normal size turn up in various species of butterflies. In the case of *P. brassicae* specimens of between 40-50 mm. wing span are referable to ab. *minor* Ksienschopolsky. According to Frohawk (1934) normal wild caught specimens vary from 63 mm. in the male to 76 mm. in the female. In the continuously brooded culture kept at the Unit of Insect Physiology the size is 55-63 mm. in the male and 54-65 mm. in the female (David & Gardiner 1961).

Occasional specimens of ab. *minor* were noted in the Cambridge stock. At times entire batches have been on the small side. Specimens bred from these were normal. It occurred to the author that ab. *minor* might be an effect of starvation rather than a genetic effect. This has been investigated as will be described.

DESCRIPTIVE

Pieris brassicae L. ab. *coerulea* ab. nov.

The normal cream and green coloration on the underside of the wings is replaced by a pale blue color. On the upper side, the white areas of the wings have a pure white appearance and are rather thinly scaled; in normal specimens they are creamy white or off white in color.

Larvae and pupae normal. The majority of the adults of this variety fail to eclose properly. Some specimens may emerge with striped scales, especially on the hind wing, which gives them a translucent blue color, deeper in shade than normally formed *coerulea*.

Holotype ♂, Allotype ♀: Two specimens selected from the F₁ generation obtained by the pairing of two ab. *coerulea* which emerged from a continuous brooded culture kept in the Laboratory since 1950. In the author's collection.

Paratypes: Five pairs also selected from the F₁. Two pairs have been deposited in the Rothschild-Cockayne-Kettlewell collection at Tring, two pairs are in the author's collection, and one pair in the collection of Mr. H. Douglas Bessemer.

Type locality: The original stock from which this aberration arose was collected in the vicinity of Cambridge, England, in 1950.

Synonyms. ab. *pallida*. Graham-Smith & Graham-Smith

ab. *anthrax*. Graham-Smith & Graham-Smith

Graham-Smith and Graham-Smith (1930) after describing *anthrax* state "Perhaps a specimen exhibited by Leeds (1909) with undersides of the hind wings a very distinct blue . . . are examples of this aberration."

Under *pallida* they state "A very marked example from Monk's Wood, Hunts., is figured by Frohawk (1914, pl. 3, fig. 20)."

The specimen figured by Frohawk (1914) is stated by him to have been taken by Mr. H. A. Leeds in Monk's Wood in 1906 and there can be little doubt that it was this specimen that was exhibited by Leeds at the Annual Exhibition of the South London Entomological and Natural History Society in 1909.

Mr. A. L. Goodson has compared the present variety with the *anthrax* and *pallida* in the R.-C.-K. collection at Tring and it in no way resembles either of them nor, in the author's opinion, does it bear any resemblance to the illustrations of *anthrax* and *pallida* in the paper by Graham-Smith and Graham-Smith (1930).

Mr. H. Douglas Bessemer, who has the original Leeds specimen in his collection, very kindly invited the author to visit him and examine it. There is no doubt that it is the same as the present variety and was wrongly identified by Graham-Smith and Graham-Smith.

Pieris brassicae L. ab. *jauni* ab. nov.

In May 1961 it was noticed that the underside of some butterflies being reared in the Laboratory was different in color to normal specimens, being a pale straw color compared to the normal greenish-yellow. The difference is more striking in freshly emerged living specimens than in dead set ones. The larvae are normal but the prepupae and pupae are a golden-yellow without any trace of the normal green. The pupae are also without the normal black speckling. Unlike some other color varieties the butterflies eclose normally.

Holotype ♂, Allotype ♀: Two specimens selected from the culture in May 1961. In the author's collection.

Paratypes: Two pairs similarly selected. In the author's collection.

Type locality: The original stock from which this aberration arose was collected at Oxford, England, in August 1959.

Pieris brassicae ab. *albinensis* Gardiner

This variety has already been described (Gardiner 1962). It is a simple recessive and is noted here since it was used with *coerulea* to obtain the double recessive.

EXPERIMENTAL

Most insects were bred according to methods already described (David & Gardiner 1952; David 1957) with the exception that when a single pair of butterflies was being paired a small nylon covered cage measuring 1 x 1 x 1½ feet was used instead of a large cage. A percentage of the larvae were also reared in 2 lb. jam jars at the rate of about 20 per jar. This enabled an accurate check to be kept on mortality. This was generally done with the F₁ broods, the larger numbers of the F₂ being reared in cages. No difficulties were encountered with either pairing or oviposition nor, apart from the F₁ of ab. *jauni*, was there any appreciable mortality of the larvae. Due to limitations of time, space and food, it was only possible to rear a small percentage of the total ova obtained. Since a single fertile *brassicae* was found to lay over 500 ova, the potential F₂ was some 125,000 individuals, an impossible number to rear.

ab. *coerulea*

In November 1962 a female emerged in the Cambridge stock. She was paired to a normal male from the same batch and the resulting ova were reared to give an F₁ of normal insects. These were crossed and a selection of the resultant ova reared.

The F_2 produced 347 normal and 116 *coerulea* butterflies, a ratio of 2.99 : 1, a very good agreement with the expected 3 : 1 ratio for a simple recessive. The majority of the *coerulea* however failed to eclose normally and remained stuck by the wing tips to the pupal case.

While this brood was being reared, further *coerulea* emerged from a batch of the stock Cambridge butterflies. Two pairings were obtained and all the F_1 and subsequent generations were *coerulea*. This aberration is therefore a recessive. It's extreme rarity under natural conditions can be explained by the difficulty the butterflies have in getting free of the pupal case.

The blue color is due to an absence of the normal yellow pigments and is present normally in the wing-membrane. This can be shown faintly by rubbing off the scales of a normal *brassicæ* between finger and thumb. A very similar appearance to *coerulea* can also be produced by immersing a normal *brassicæ* in the bleaching agent sold, under the name of 'Parazone.' The question of the chemistry of this and other color forms of *brassicæ* is at present under investigation by a colleague of the present author and will be dealt with in a later paper.

The failure to eclose is more marked in the males than the females. In the first two generations only about two per cent of the specimens could be described as immaculate. About 50 per cent fail to clear the pupal case and are stuck by one or both fore wing tips; others get clear but one or more wings fail to expand. In many instances there is extensive stripping of the scales, especially of the hind wing, which then has a deep blue translucent appearance.

After several generations the percentage of normal eclosions rises to about 80 per cent. This is similar to the trend that occurred in *albinensis* (Gardiner 1962). Since 1961 *albinensis* has been passed through a further ten or twelve generations and the percentage failing to eclose normally is now negligible, and there seems little doubt that *coerulea* will eventually reach this stage.

While failure to eclose is a semi-lethal factor linked, initially, with *coerulea* it is also partially controlled by the temperature at which eclosion takes place. Two experiments were done on this, using the F_2 generation of the pure line in the first and *albinensis* in the second.

A cage of *coerulea* pupae was kept at 20°C. until 12 normal (5.7%) and 198 crippled specimens had emerged. The remaining pupae were than transferred to 12.5°C. and at this temperature 38 normal (27.0%) and 103 crippled specimens emerged.

In the second experiment *albinensis* was used, half the pupae being at 25°C. and half at 12.5°. At 25°C. 15 normal (30.0%) and 35 crippled emerged while at 12.5°C. 28 normal (58.4%) and 20 crippled emerged.

These results clearly show that failure to eclose is more marked at higher temperatures.

ab *jauni*

These were noted in May 1961 in a stock obtained from Oxford which had been bred for a number of generations in the Laboratory.

A pair of these butterflies was mated and 100 of the ova reared. In this generation the mortality, mainly granulosis virus, was 76%. The 14 adults obtained were all of the variety. These were paired at random and 100 F₂ ova reared to produce 97 butterflies, also of the variety.

After the F₁ males had paired with their sisters they were paired with normal females and a percentage of the ova laid were reared to produce all normal butterflies. These were mated at random to produce the F₂ of which a percentage of the total ova laid were reared, with little mortality. This generation produced 100 normal and 34 ab *jauni* butterflies. This is a 3 : 1 ratio.

This variety seems to be due to the lack of the normal greenish colored pigment. It segregates as a simple recessive and breeds true. It is of interest as being one of the few known instances in the butterflies in which a single gene manifests its effect not only in the adult but also in the earlier stages.

ab *minor*

About 200 final instar larvae, which had been kept well supplied with food during their earlier stages, were allowed to feed normally for 3 days and were then starved. About half died, the remainder wandered around their cage and eventually pupated. Normally, at 20°C, the final instar larvae feeds for 5 days before pupation (David & Gardiner, 1962).

The majority of the pupae produced butterflies, all ab *minor*, the smallest specimens having a wing span of 37 mm. male, 38 mm. female.

As a control siblings to the starved larvae were kept and allowed to feed normally and pupate when ready. These all produced normal sized butterflies.

Eggs obtained by the pairing of several of these *minor* were reared and an adequate supply of food was given throughout their life. Normal size specimens were produced.

It is evident from this result that ab *minor* can be produced by a condition of the external environment, starvation, on the larvae. Nevertheless the size of *brassicae* is, at least in some instances, genetically controlled. This will be dealt with more fully in the discussion.

ab *nigroviridescens*

About a hundred prepupae of the Cambridge stock were put in a desiccator containing water to give 100% R.H. and kept at 20°C. One reasonable specimen of *nigroviridescens* Rocci emerged and two partially crippled adults intermediate between normal and *nigroviridescens*.

No breeding was done from these nor have any similar specimens ever been noted in the Cambridge stock. It is considered probable, however, that the *nigroviridescens* produced was a result of the saturated humidity of the environment.

ab *coerulea/albinensis*

The cross between *coerulea* and *albinensis* was performed both ways. One brood, *coerulea* ♂ X *albinensis* ♀ being reared by the author and the brood from the reciprocal cross being reared by Mr. C. F. Rivers of the Agricultural Research Council's Virus Research Unit.

In both crosses the butterflies used were from a pure bred line of the respective recessive.

In both crosses the several hundred individuals of the F₁ were all normal *brassicae*.

The F₂ of both crosses gave a ratio very close to 9 : 3 : 3 : 1 as is to be expected from the independent assortment of two pairs of allelomorphs. The back-cross was also performed to give a 1 : 1 : 1 : 1 ratio. The numbers involved are shown in Table 1.

These results show that linkage, which has not yet been shown to occur in butterflies (Ford 1946) is not present. Since *brassicae* has a relatively large number, 15, of chromosomes (Lorković, 1941) this is perhaps not very surprising. It would have been more interesting to have crossed *coerulea* with *jauni*, since both involve a change in the same pigment, unlike *albinensis* which is caused by an absence of black. Unfortunately by the time *coerulea* appeared the *jauni* form had been lost.

Cross	normal	<i>coerulea</i>	<i>albinensis</i>	<i>albinensis</i> <i>coerulea</i>
<i>coerulea</i> ♂ X <i>albinensis</i> ♀	921	295	304	97
<i>albinensis</i> ♂ X <i>coerulea</i> ♀	695	233	227	76
<i>coerulea/albinensis</i> X double heterozygote	97	96	92	95

Table 1. The numbers of butterflies reared in the F₂ of the double recessive cross and the F₁ of the back-cross.

As might be expected the majority of the double recessives failed to eclose normally but, like their respective grandparents, the percentage of normal eclosions rises with continual breeding.

Homeosis

This is extremely rare in butterflies. In *brassicae* specimens are known in which the fore wing black markings are duplicated on one side of the hind wing. According to Ford (1946) there is no definite evidence of its nature in butterflies but it has been shown to be genetic in *Drosophila*.

During the course of hybridising Cambridge stock *brassicae* with race *cheiranthi* from the Canary Islands, three homeotic specimens turned up in one brood of some 300 individuals, all the progeny of one pairing.

The male parent was an F_3 from a back-cross of an F_1 *brassicae/cheiranthi* hybrid to *cheiranthi*, while the female was an *albinensis* extracted in the F_2 of an *albinensis* crossed to the F_1 *brassicae/cheiranthi* hybrid. The *cheiranthi* used for the back-cross and the F_1 *brassicae/cheiranthi* hybrids had the same father.

All the homeotics were male with the black markings on the fore wing underside partially replicated on the hind wing of one side only.

One male was mated to his sister and sib pairing was continued to the F_3 , some 300 individuals being reared in each generation. No more homeotic specimens were produced.

This is of course a negative result, but it shows that homeosis is not caused by a single recessive allelomorph. The most likely explanation of its occurrence, in this instance, is an upset in the genetic compatibility due to a slight variation between the parent genes.

DISCUSSION

The continuously brooded stock of *brassicae* kept at the Unit of Insect Physiology is now thirteen years old and provides a unique opportunity for the study of variation in a butterfly. The numbers produced have varied from a few dozen to a thousand or more per week.

In addition to a number of known aberrations two new ones, *jauni* and *albinensis*, have been produced. A third, *coerulea*, was only previously known from two examples, both taken over fifty years ago.

As was to be expected, changes in the color of the wings, *coerulea* and *jauni*, are recessives. It is probable that the yellow form *ab flava* Kane is also recessive and its extreme rarity is, like *coerulea*, due to failure to eclose normally.

The failure to eclose of *coerulea* and *albinensis* appears to be due to the wings sticking to the pupal case. Indeed scales can be seen to be left behind in the pupal cases from which specimens have succeeded in getting free. It is however a disability which is overcome by con-



Left side, from top down. *ab coerulea* ♀ allotype (upperside). ♀ para-type (underside L). ♂ holotype (underside). ♀ *coerulea/albinensis* (upperside) L. Right side. Typical *brassicæ* ♀ (underside). *ab jauni* ♂ holotype (underside). *ab minor* ♀ (upperside). ♂ *brassicæ/cheiranthi* hybrid underside showing homeosis.

tinued breeding. This failure to eclose, however, again occurs if *albinensis*, from a normally emerging stock is crossed to typical *brassicæ* and extracted again in the F_2 . The effect is mechanical, more marked in the males than in the females and is affected by temperature. It might be that it is a genetic effect and it would be interesting to breed from the crippled specimens if this were possible. Since only perfect images are used for breeding, there is a very strong selection towards normality and only recessive genes producing crippling are rapidly eliminated for butterflies which cannot fly are unable to mate or oviposit.

Although *minor* can be produced by starvation the size of *brassicæ* is also genetically controlled. From time to time small backward larvae are found in stocks of *brassicæ*. Attempts to breed these have so far been unsuccessful. Usually the larvae die. Two or three have been brought to pupation, the pupae being comparable in size to *minor*, but no adults emerged.

In 1951 the wing span of the Cambridge stock *brassicæ* was 53 mm. ♂ and 58 mm. ♀ (David & Gardiner, 1952). By 1960 however this had risen to 58.5 mm. in the male, while the females had decreased to 57.9 mm. (David & Gardiner, 1961).

The continuous inbreeding of the Cambridge stock has produced specimens with virtually no size difference between the sexes. This is smaller, especially in the female than the respective male and female sizes of 63 mm. and 76 mm. given by Frohawk (1934). It would appear that Frohawk had some unusually large female specimens before him. The author now believes that this figure may be a misprint in Frohawk's book. A 76 mm. *brassicæ* would be a giant specimen. Wild specimens caught in 1943 from the author's collection being 58 mm. and 63 mm, while F_1 bred examples from a freshly caught wild female in 1957 are 58 mm. in both sexes.

The Cambridge stock is therefore not significantly different in size to some wild populations, but both it and wild populations vary in size over a period of years.

In *P. brassicæ* race *cheiranthi* the males are 60-65 mm. and the females 65-70 mm. (12 examples measured), but the wings are also broader so that the butterfly looks even larger. If these are crossed to Cambridge stock *brassicæ* the F_1 insects are intermediate in size and in the F_2 a range of sizes are produced. Size in this instance is clearly under genetic control.

The Cambridge stock *brassicæ* are on the whole lighter and with less black than wild populations. The varieties that have turned up, *reducta* Fritsch, *colliurensis* Gelin and intermediates, are all those with a reduction of black.

The author has in his collection a female series from heavily marked with a trace of *fasciata* Kiefer to extreme *colliurensis*, in which the remaining spot is very feint.

The evidence from this and also from hybrid crosses to *cheiranthi* (which will be dealt with fully in a subsequent paper) clearly shows that a multifactorial effect is involved in the degree of black on the wings.

SUMMARY

Two new varieties of *Pieris brassicae* L., *coerulea* and *jauni* are named and shown to be recessive in character.

The failure to eclose in early broods of *coerulea* and *albinensis* is shown to be partly influenced by temperature.

The double recessive *coerulea/albinensis* has been reared and the two allelomorphs concerned shown not to be linked.

Ab. nigroviridescens is considered to be probably produced by saturated humidity.

The small form, *minor*, can be produced by starvation. Nevertheless size in *brassicae* is also genetically controlled.

The size of *brassicae*, both bred and wild, shows variation over a period of years. It is also believed that Frohawk's figure of 76 mm. for the female wing span is a misprint.

Homeosis is shown not to be due to a simple recessive gene.

The Cambridge stock of *brassicae* shows a tendency for reduction in the amount of black on the wings. The evidence is that this is multifactorial.

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